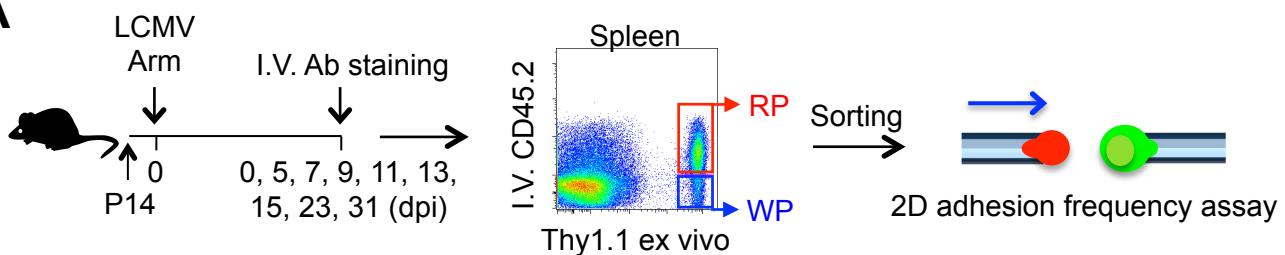
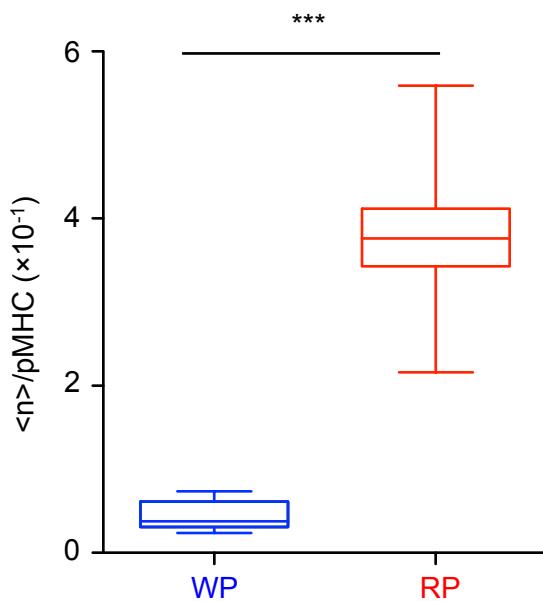
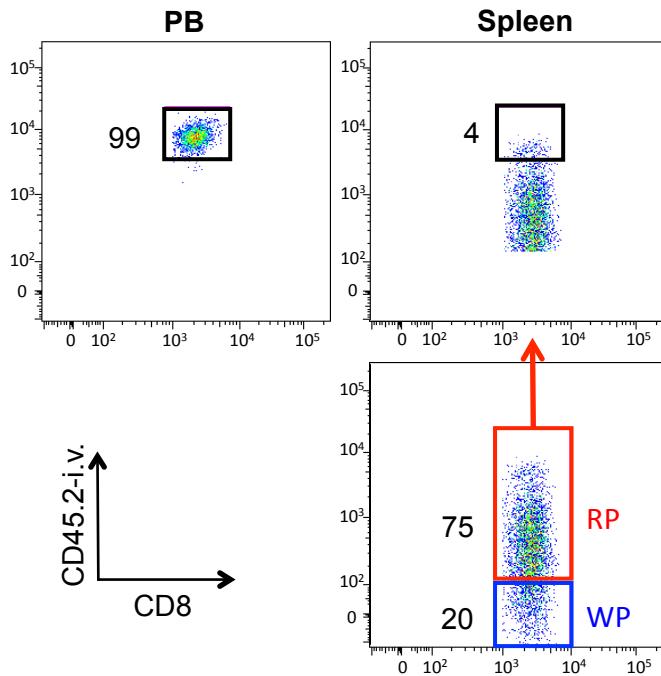


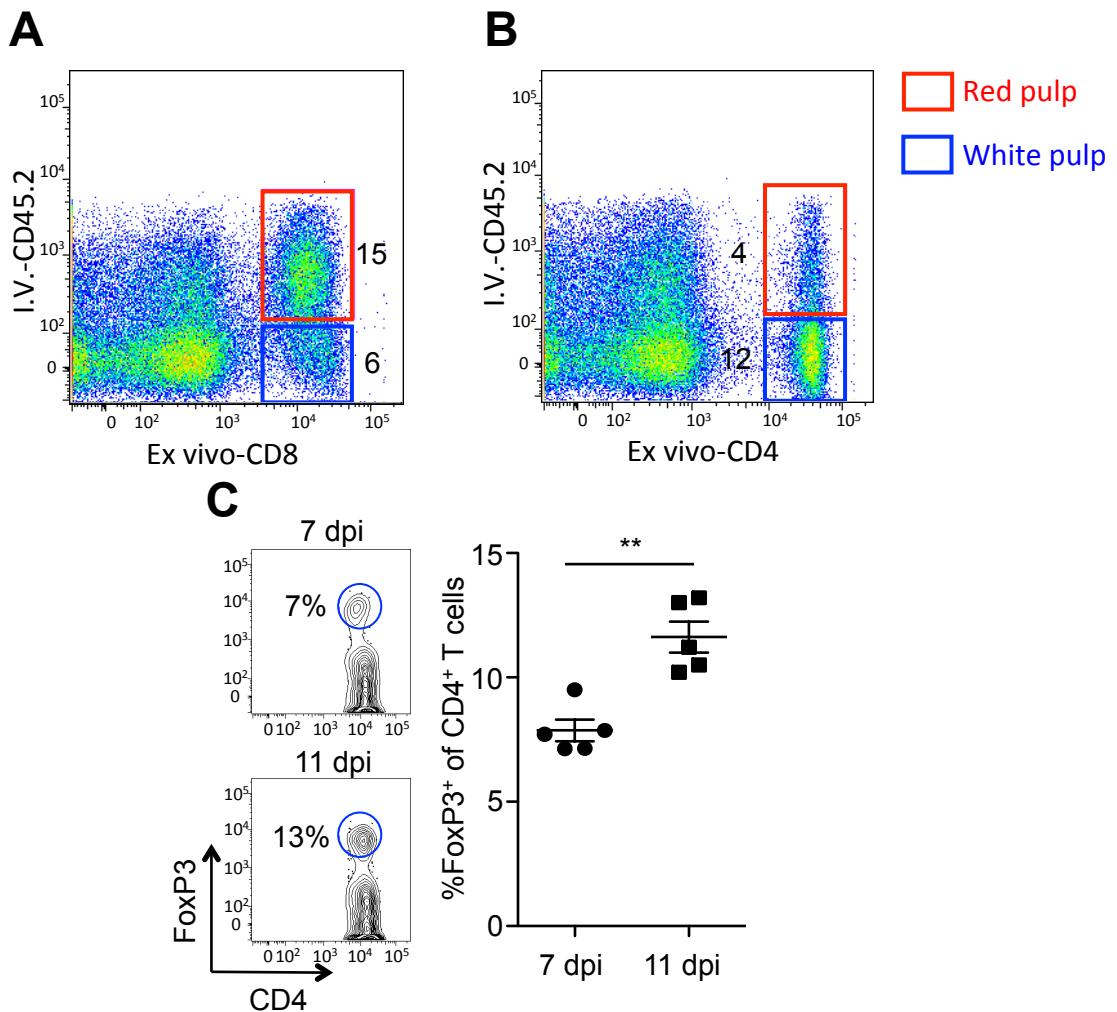
**Fig. S1****A****B**

**Figure S1. CD8<sup>+</sup> T cells from RP display higher bond formation of TCR-pMHC-CD8 interaction than those from WP, Related to Figure 1.** **A.** Experimental scheme for Fig. 1A. **B.** Mice that received P14 T cells were infected with LCMV-Arm 11 days. Normalized adhesion bonds (average number of bonds per pMHC site density) of TCR-pMHC-CD8 tri-molecular interaction for P14 T cells from WP and RP were obtained from micropipette assay. \*\*\*= $P < 0.001$ .

**Fig. S2**

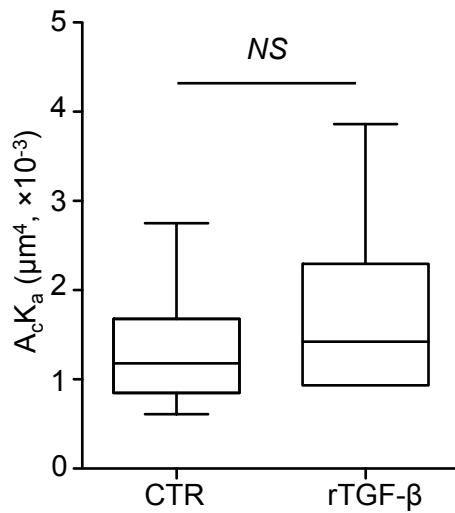


**Figure S2. RP compartment separated by intravascular cell staining contains only few intravascular cells, Related to Figure 2.** CD8<sup>+</sup> T cells were isolated from PB and spleen of mice that were infected with LCMV-Arm at 11 dpi.

**Fig. S3**

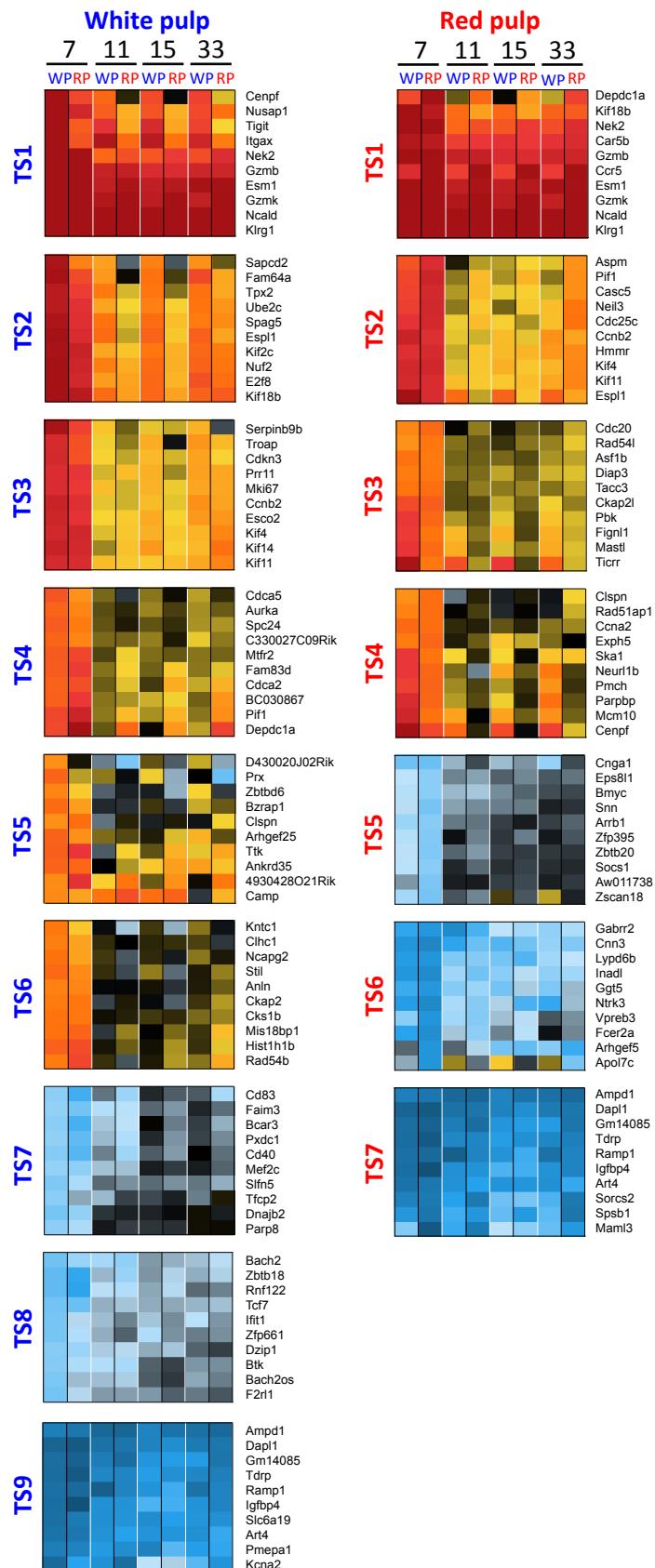
**Figure S3. Anatomic location of T cells and the frequency of FoxP3<sup>+</sup> CD4<sup>+</sup> T cells in spleen. Related to Figure 3.** A, B. Splenic anatomic location of CD8<sup>+</sup> (A) or CD4<sup>+</sup> T cells (B) was analyzed by intravascular staining at 11 dpi. C. At 7 and 11 dpi, percentages of FoxP3-expressing CD4<sup>+</sup> cells were analyzed by flow cytometry. \*\* =  $P < 0.01$ .

**Fig. S4**

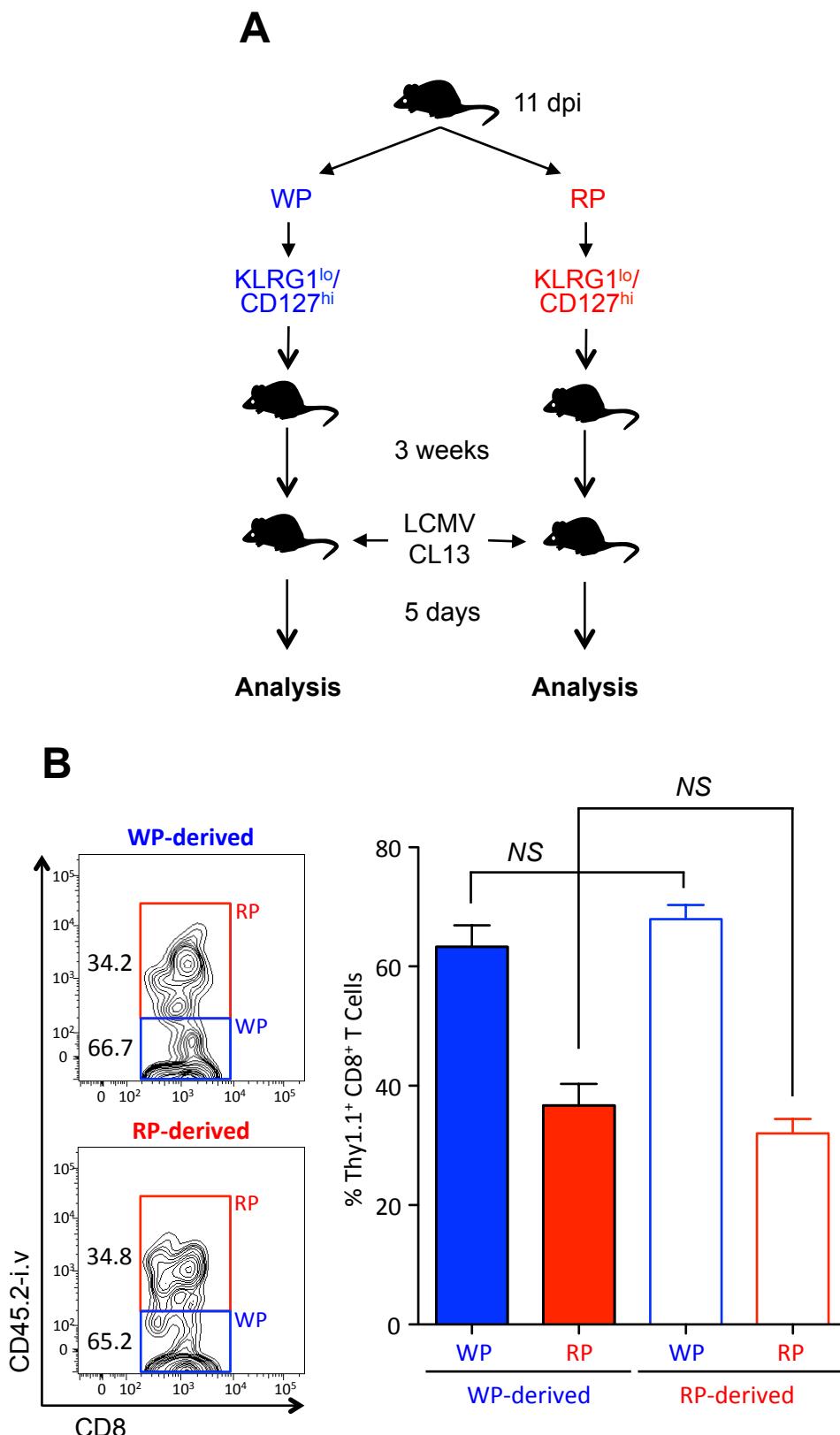


**Figure S4. Recombinant TGF- $\beta$  treatment does not change effective 2D TCR affinity of CD8 $^{+}$  T cells at 7 dpi, Related to Figure 3.** Mice that received P14 T cells were infected with LCMV-Arm for 7 days. P14 T cells from spleen were sorted and either untreated or treated with rTGF- $\beta$  for 24 hrs in the presence of recombinant IL-2. The effective 2D TCR affinity was measured by micropipette assay. NS=not significant.

**Fig. S5**



**Figure S5. Anatomic segregation regulates gene expression patterns of CD8<sup>+</sup> T cells, Related to Figure 4.** Top 10 DEGs for each TS in Fig. 4B were selected base on fold change at 7 dpi and shown in heat map.

**Fig. S6**

**Figure S6. Both the WP- and RP-derived memory T cells are preferentially enriched in the WP.** Related to Fig. 6. A. Experimental scheme for Fig. 6C. B. Mice that received P14 T cells were infected with LCMV-Arm for 11 days. 500,000 Memory precursor P14 T cells (KLRG1<sup>lo</sup>/CD127<sup>hi</sup>) from the splenic WP or RP were sorted and adoptively transferred into naïve mice. Three weeks later, locations of cells were analyzed by flow cytometry following intravascular cell staining. NS=not significant.

**Table S1**

Figure	Dpi	Splenic location	2D TCR affinity ( $A_cK_a$ , $\mu\text{m}^4$ )		TCR site density (mol/ $\mu\text{m}^2$ )	pMHC site density (mol/ $\mu\text{m}^2$ )
			Mean	SEM		
1B	0	WP	1.33E-3	2.30E-4	84	6
		RP	1.57E-3	2.73E-4	85	6
	5	WP	9.86E-4	1.44E-4	43	24
		RP	9.03E-4	8.86E-5	47	24
	7	WP	1.54E-3	2.29E-4	42	15
		RP	1.54E-3	1.42E-4	43	15
	9	WP	1.46E-3	1.10E-4	47	18
		RP	4.67E-3	5.08E-4	44	6
	11	WP	2.17E-3	1.48E-4	44	13
		RP	1.01E-2	1.03E-3	39	5
	13	WP	2.89E-3	4.71E-4	34	8
		RP	3.24E-3	7.66E-4	32	8
	15	WP	1.06E-3	9.92E-5	33	32
		RP	9.02E-4	1.68E-4	31	32
	23	WP	4.96E-4	7.85E-5	46	46
		RP	4.42E-4	4.80E-5	41	46
	31	WP	6.31E-4	7.62E-5	38	41
		RP	6.39E-4	7.67E-5	32	41
1D	11	WP	1.38E-3	2.10E-4	41	6
		RP	4.60E-3	3.71E-4	43	9
2A	7	WP	1.29E-3	2.15E-4	48	11
		RP	1.43E-3	1.21E-4	49	11
		PB	1.41E-3	1.36E-4	39	14
	11	WP	3.80E-3	4.56E-4	19	9
		RP	1.03E-2	1.11E-3	20	4
		PB	1.31E-2	1.21E-3	16	4
	15	WP	1.21E-3	1.25E-4	52	11
		RP	1.30E-3	1.52E-4	51	11
		PB	1.61E-3	2.05E-4	36	11
2B	11	WP-GP33	2.02E-3	2.02E-4	56	4
		RP-GP33	4.33E-3	4.79E-4	54	4
		WP-GP35A	5.60E-4	6.02E-5	56	23
		RP-GP35A	1.04E-3	9.45E-5	54	23

**Table S1 Continued**

Figure	Dpi	Splenic location	2D TCR affinity ( $A_c K_a, \mu\text{m}^4$ )		TCR site density (mol/ $\mu\text{m}^2$ )	pMHC site density (mol/ $\mu\text{m}^2$ )
			Mean	SEM		
3A	11	CTR WP	3.84E-3	3.10E-4	16	16
		CTR RP	1.45E-2	1.41E-3	18	4
		CD4 $\Delta$ WP	1.52E-2	1.46E-3	19	4
		CD4 $\Delta$ RP	1.40E-2	9.22E-4	21	4
3B	11	WT WP	1.85E-3	2.47E-4	26	18
		WT RP	5.04E-3	8.42E-4	24	8
		FoxP3-DTR WP	3.76E-3	3.28E-4	27	8
		FoxP3-DTR RP	4.55E-3	3.89E-4	24	8
3C	11	CTR WP	1.37E-3	1.92E-4	60	10
		CTR RP	5.69E-3	1.47E-3	51	3
		SB431542 WP	7.74E-3	1.18E-3	45	3
		SB431542 RP	9.98E-3	1.32E-3	40	3
3D	11+1	CTR RP	2.97E-3	3.32E-4	57	5
		TGF- $\beta$ RP	1.64E-3	2.02E-4	43	14
3E	10+1	CTR	2.00E-3	2.48E-4	57	11
		FoxP3-	1.96E-3	2.80E-4	57	14
		FoxP3+	1.10E-3	1.81E-4	57	11
		TGF- $\beta$ Abs	1.73E-3	2.05E-4	58	11
4D	11	CTR WP	2.59E-3	2.68E-4	45	9
		CTR RP	8.15E-3	1.31E-3	51	2
		Nystatin WP	1.12E-3	1.54E-4	48	19
		Nystatin RP	1.23E-3	1.30E-4	47	19
4E	11	CTR WP	1.86E-3	2.27E-4	35	14
		CTR RP	4.18E-3	4.76E-4	34	5
		CO WP	1.42E-3	1.36E-4	48	14
		CO RP	2.02E-3	1.98E-4	33	14
5B	11	Effector WP	7.55E-4	7.53E-5	38	19
		Memory WP	7.61E-4	1.27E-4		19
		Effector RP	1.29E-3	1.89E-4	37	9
		Memory RP	2.52E-3	2.80E-4		19

**Table S1. Summary of mean and SEM for effective 2D TCR affinity, Related to Figure 1.** Values for effective 2D TCR affinity shown in figures are summarized along with the TCR and pMHC site densities used for their calculation.

**Table S2**

#	GO Term	Name	RP		WP	
			Gene Size	B-H P value	Gene Size	B-H P value
<b>Lymphocyte Effector Response</b>						
1	GO:0048584	Positive Regulation Of Response To Stimulus	42	7.65E-09	15	1.39E-01
2	GO:0002703	Regulation Of Leukocyte Mediated Immunity	22	2.65E-06	11	1.85E-01
3	GO:0002706	Regulation Of Lymphocyte Mediated Immunity	21	3.80E-06	11	1.34E-01
4	GO:0002697	Regulation Of Immune Effector Process	23	2.92E-05	12	2.26E-01
5	GO:0002705	Positive Regulation Of Leukocyte Mediated Immunity	15	1.18E-04	6	6.13E-01
6	GO:0002699	Positive Regulation Of Immune Effector Process	15	4.96E-04	6	7.15E-01
<b>Regulation of immune response</b>						
1	GO:0002684	Positive Regulation Of Immune System Process	53	1.63E-13	24	6.92E-02
2	GO:0050778	Positive Regulation Of Immune Response	37	1.50E-09	12	7.57E-01
3	GO:0002253	Activation Of Immune Response	20	2.71E-04	6	9.87E-01
<b>Regulation of lymphocyte activation</b>						
1	GO:0050865	Regulation Of Cell Activation	32	4.77E-06	22	2.61E-02
2	GO:0002694	Regulation Of Leukocyte Activation	31	1.20E-05	21	3.94E-02
3	GO:0050867	Positive Regulation Of Cell Activation	23	7.92E-05	14	1.55E-01
4	GO:0002696	Positive Regulation Of Leukocyte Activation	22	1.91E-04	13	2.35E-01
5	GO:0051251	Positive Regulation Of Lymphocyte Activation	21	2.70E-04	13	1.91E-01
6	GO:0051249	Regulation Of Lymphocyte Activation	27	2.73E-04	20	4.06E-02
<b>Response to Bacteria</b>						
1	GO:0009617	Response To Bacterium	28	4.07E-04	14	6.80E-01
<b>Regulation of cytokine production</b>						
1	GO:0001817	Regulation Of Cytokine Production	37	2.38E-09	15	3.73E-01
2	GO:0001819	Positive Regulation Of Cytokine Production	21	1.79E-06	9	3.80E-01
<b>Endocytosis</b>						
1	GO:0006897	Endocytosis	31	5.30E-04	15	8.00E-01
<b>Immune response regulating signaling transduction</b>						
1	GO:0002764	Immune Response-Regulating Signal Transduction	15	4.22E-04	7	6.03E-01
<b>Antigen processing and presentation</b>						
1	GO:0048002	Antigen Processing And Presentation Of Peptide Antigen	18	4.35E-08	10	2.24E-02
2	GO:0019882	Antigen Processing And Presentation	22	9.87E-05	15	3.79E-02
<b>Regulation of adaptive immune response</b>						
1	GO:0002819	Regulation Of Adaptive Immune Response	17	2.52E-04	9	2.70E-01
<b>Lymphocyte activation</b>						
1	GO:0001775	Cell Activation	51	7.91E-10	34	1.29E-03
2	GO:0045321	Leukocyte Activation	46	3.26E-09	28	1.94E-02
3	GO:0046649	Lymphocyte Activation	38	7.21E-07	24	4.14E-02
4	GO:0042110	T Cell Activation	24	2.08E-04	15	1.79E-01
<b>Immune response</b>						
1	GO:0006955	IMMUNE RESPONSE	92	1.96E-16	62	5.57E-07
2	GO:0006952	DEFENSE RESPONSE	73	2.54E-09	38	2.31E-01
3	GO:0006954	INFLAMMATORY RESPONSE	46	6.67E-09	22	2.77E-01
4	GO:0009611	RESPONSE TO WOUNDING	60	7.12E-09	37	3.04E-02
5	GO:0045087	INNATE IMMUNE RESPONSE	22	5.15E-04	7	9.89E-01

**Table S2.** Lists of GO term, gene size, B-H P value from enrichment analysis of biological processes. Related to Figure 4.